Detailed Saccharification of Washed Corn Fiber

Tammy Kay Hayward
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Experiment Run Dates: May 31 to June 9,1995
Researcher: Tammy Kay Hayward
Lab Book References: #1651, 011-013

Objectives: Based on the limited data found in previous enzyme loading experiments, collect more detailed data on saccharification of corn fiber. Examine the cellulose conversion in washed corn fiber solids in detail and close the mass balance. To examine the kinetics of oilgomeric and monomeric sugar release with this substrate. To examine the release of five carbon sugars and possible xylanase activity. To provide data for modeling.

Materials and Methods:
In order to close the mass balance, a dummy time zero flask was prepared to collect starting data, A calculation was performed to determine what the initial solids loading should be in order to have enough residual solids at time find to analyze. The CAT task requires 30-50 mL per time point to test for oligomeric sugars. Twenty time points would be taken and the volume of the saccarification had to account for this as well. A Bioflow III reactor will a 2 kilogram working slurry weight was used.

Corn Fiber Solids-Just prior to the reactor experiment, fresh pretreated corn fiber was diluted in D.I. water, adjusted to pH 5 with lime and then filtered onto a paper filter under vacuum. The wet solid cake was then washed extensively with water until the filter liquid was colorless. (liquor starts as a dark brown). The goal of this was to remove the glucose and other free sugars from the substrate and create a solid similar to the one used in the enzyme loading experiment. The wet washed solids cake was placed in a plastic bag and kneaded into a homogenous yellow corn clay. Left-over solids were frozen. The saccharification was performed at 5% solids, The solids and CSL were autoclaved prior to use. Antibiotics were not used.

Cellulase Enzyme: The cellulase enzyme used in the experiments was CPN PDU lot with a measured Filter Paper Activity of 70 units per mL. The beta-glucosidase activity is 231 units per milliliter per minute. Both activities were measured on the filter sterile enzyme by Bill Adney. The enzyme was diluted twenty-fold in sterile D.I. water prior to use. No precipitate formed with this preparation. The undiluted cellulase is suspended in 280 g/L sucrose. The vessel attempted to duplicate results from the first saccharification experiment in enzyme loading study and used 7 FPU/g cellulose (88 mL of 1/20 CPN to the vessel and 8.8 mL to the time zero flask)

Media: Corn Steep Liquor from Grain Products Corporation was diluted ten-fold in D.I. water, adjusted to pH 5 with ammonium hydroxide, autoclaved for 30 minutes and then filter sterilized. The washed ECF solids were suspended in 0.5% CSL for pH control in saccharification. This low level improves the resolution of glucose and cellobiose peaks on the HPLC.

Conditions: The vessel was operated at 34°C and 150 rpm. Initial samples were taken from a side port until the level dropped below the port and the solids went into slurry, at which time a suction bottom draw port was used. Forty milliliter samples were taken once a hour for the first 10 hours, then daily for eight days. All samples were boiled immediately after sampling in sealed sterile

centrifuge tube to denature the enzyme. Each sample was then chilled and centrifuged. The YSI glucose and pH were recorded. The solids were separated from the liquids and both were frozen to prevent spoilage. All of the samples were submitted to the CAT task at the end of the experiment.

Results:

During the experiment, samples were run on the YSI for glucose. As expected, glucose concentration increased over time (see figure 1). The pH of the vessel was also monitored off-line. The pH did drop from 4.98 to 4.63 based on the pH meter. The weak buffer of only 0.5% CSL in water may have allowed this slight pH change in the solution over the 192 hours of saccharification, or the pH meter may have contributed some variation. The slurry was observed under the microscope, no organisms were present. A milliliter of slurry was spread on a YPD plate and incubated for one week at 30 C, no colonies formed.

Some time after the experiment was completed, the CAT task completed the HPLC analysis on August 25th The results from the HPLC CAT task glucose are compared with the YSI glucose obtained at the time of the experiment. The HPLC is more variable at the early time points and slightly higher at the later time points than the YSI data (see figure 2).

The CAT task also performed the 4% acid hydrolysis for each sample. The total glucose after hydrolysis minus the monomeric glucose was called oligomeric glucose. If the oligomeric glucose is plotted against the cellobiose concentrations, there seems to be no difference between these values (see figure 3). The HPLC analysis also included xylose, arabinose, galactose and mannose. The total sugars, after 4% acid hydrolysis are shown as sugar release over time in figure 4. The only sugar which increases during enzymatic saccharification is glucose.

The Excel spreadsheet was used to try to perform a carbon balance on this saccharification. The initial and final solids and liquids were analyzed by the CAT task. According to the spreadsheet a cellulose conversion of 97.6% is obtained. That number is still in doubt however because the glucose balance is a 19.43% error and the carbon recovery is only at 80.66%. The CAT task also performed CHN analysis on both the initial and final solids and liquids. The carbon balance based on CHN is very poor at 171.14% conversion. Hydrogen and nitrogen balances are similarly poor (see figure 5).

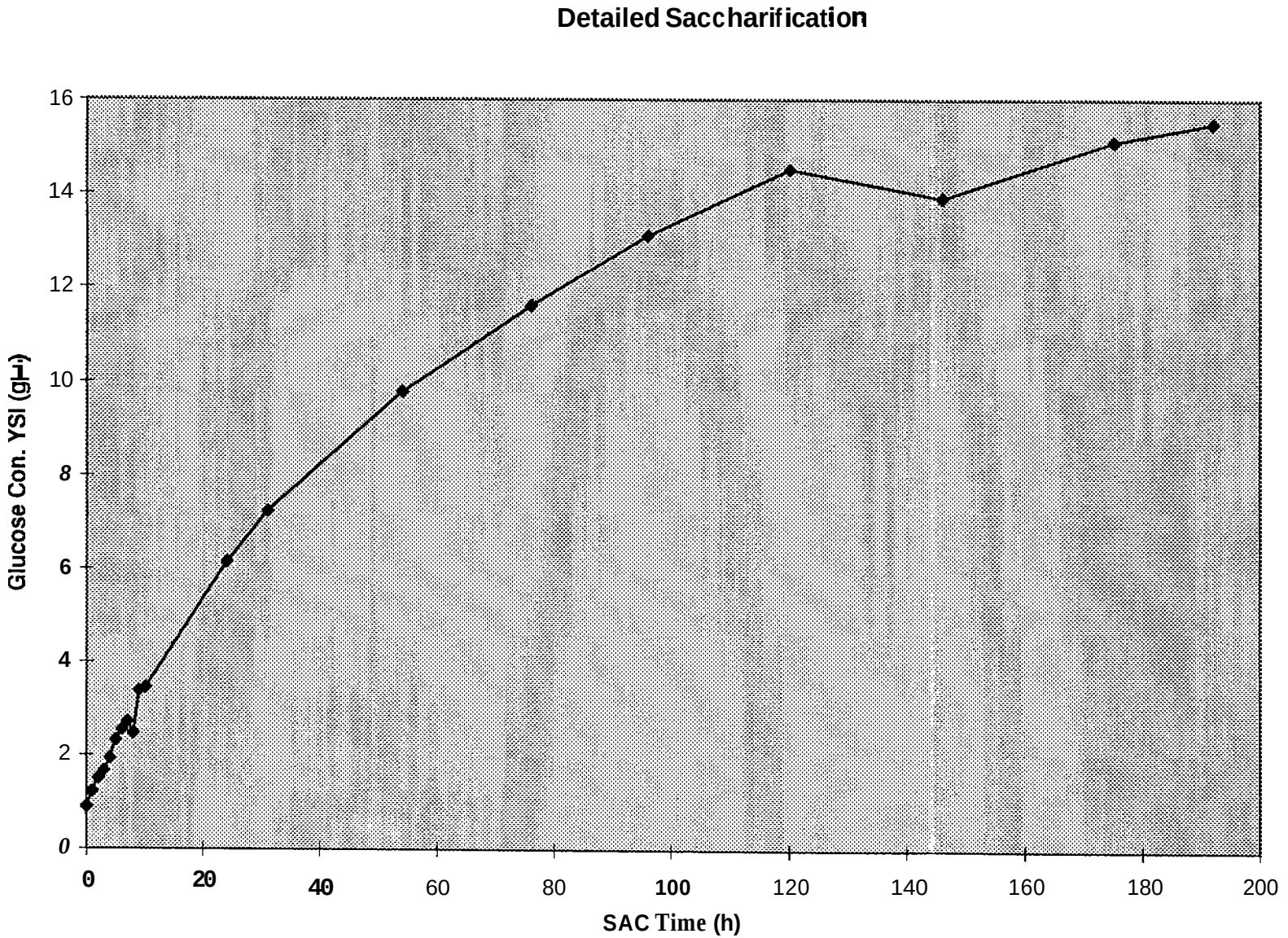
Conclusions:

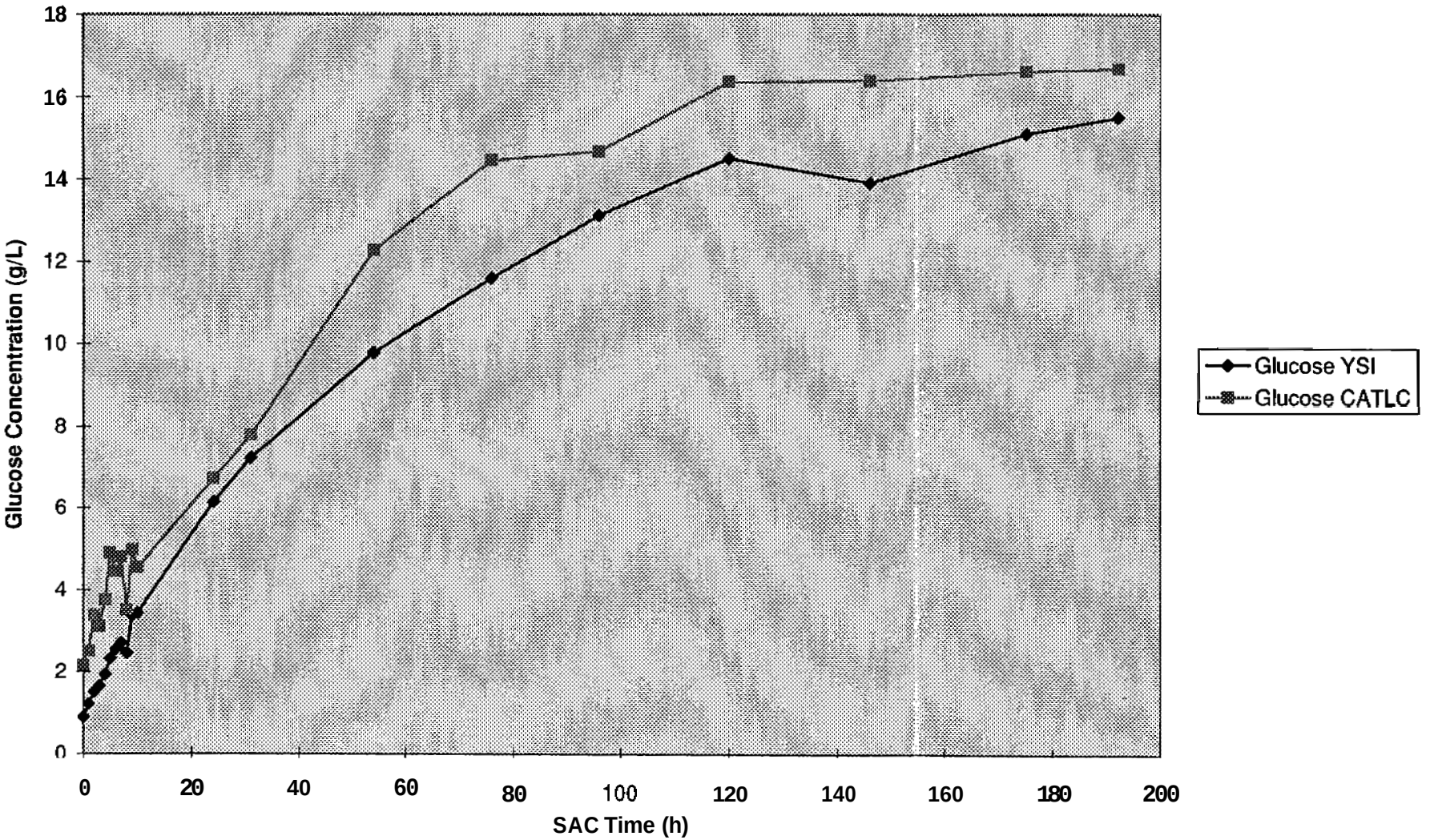
Detailed data for the kinetics of saccharification of washed corn fiber solids at 7 FPU per gram of cellulose and 5% solids was provided by this experiment. Samples were taken every hour for the first 8 hours, and then up to 192 hours. This data can be used for modeling. All the necessary data was collected for closing the mass balance. That data was entered into the Excel Carbon Balance spreadsheet. The carbon recovery was only 80.66%. Significant lack of mass closures on several items occurs based on the analysis numbers for solids and liquids. The cellulose conversion number of 97.6% cannot be confirmed by such a poor mass closure. It was hypothesized that the corn fiber solids might release unusual oligomeric sugars during enzymatic hydrolysis. The only oligomeric glucose released during saccharification was cellobiose, which is expected during cellulosic breakdown. As for xylanase activity, there was no detectable, significant change in the mount of xylose either monomeric or oligomeric during saccarification, suggesting that there is no xylanase activity under these conditions.

Figure 1: Glucose release over time
Figure 2: Comparison of CATLC and YSI glucose
Figure **3:** Glucose Oligomers **and** Cellobiose
Figure 4: Sugar Release over **time**
Figure 5: SAC Carbon Balance Sheet

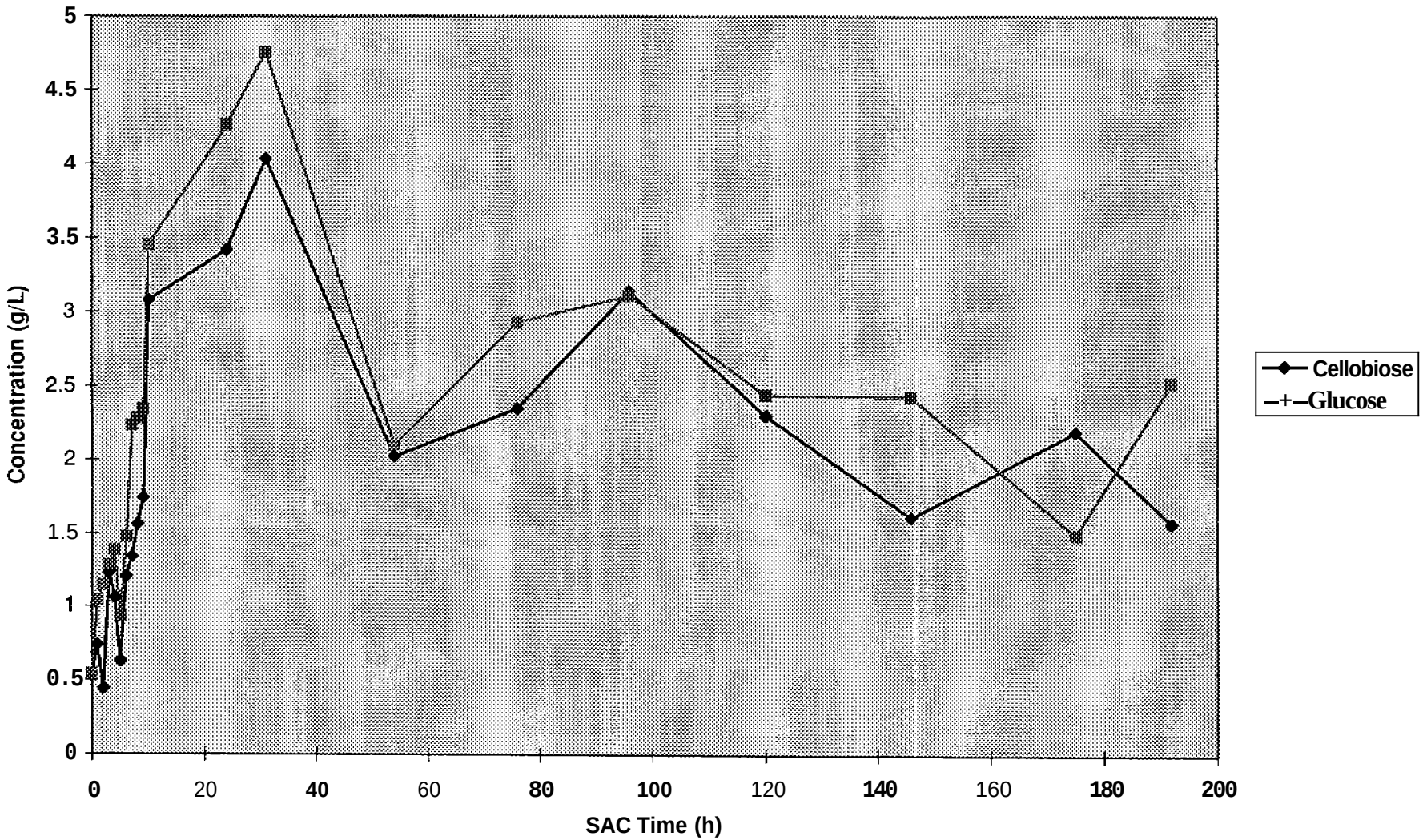
Appendix CAT **task** reports 95-090, 95-089, 95-095, 95-106

	Detailed Saccharification																		
	CAT#95-089								Total Sugars						Oligomers				
SAC Samp	SAC Time	Glucose Y	Cellobiose	Glucose C	Xylose	Galactose	Arabinose	Mannose	Cellobiose	Glucose	Xylose	Galactose	Arabinose	Mannose	Cellobiose	Glucose	Xylose	Galactose	Arabinose
0	0	0.902	0.54	2.16	0.58	0.07	0.46	0	0	2.7	1.11	0.03	0.38	0		0.54	0.53	-0.04	-0.08
1	1	1.22	0.74	2.52	0.53	0.13	0.42	0	0	3.56	1.52	0.35	0.78	0		1.04	0.99	0.22	0.36
2	2	1.51	0.44	3.37	0.57	0.15	0.43	0	0	4.51	1.67	0.38	0.8	0		1.14	1.1	0.23	0.37
3	3	1.66	1.23	3.12	0.64	0.17	0.48	0	0	4.4	1.48	0.33	0.72	0		1.28	0.84	0.16	0.24
4	4	1.94	1.06	3.74	0.63	0.16	0.46	0	0	5.12	1.66	0.35	0.78	0		1.38	1.03	0.19	0.32
5	5	2.33	0.63	4.89	0.71	0.19	0.5	0	0	5.82	1.71	0.37	0.77	0		0.93	1	0.18	0.27
6	6	2.55	1.2	4.44	0.71	0.19	0.52	0	0	5.91	1.63	0.37	0.77	0		1.47	0.92	0.18	0.25
7	7	2.72	1.34	4.8	0.75	0.12	0.47	0	0	7.03	2.2	0.82	1.21	0		2.23	1.45	0.7	0.74
8	8	2.47	1.56	3.5	0.65	0.17	0.5	0	0	5.78	1.64	0.24	0.7	0		2.28	0.99	0.07	0.2
9	9	3.38	1.74	4.97	0.75	0.18	0.5	0	0	7.31	1.78	0.27	0.73	0		2.34	1.03	0.09	0.23
10	10	3.44	3.08	4.54	0.84	0.15	0.5	0	0	7.99	2.13	0.7	1.04	0		3.45	1.29	0.55	0.54
11	24	6.14	3.42	6.71	0.79	0.07	0.41	0	0	10.97	1.54	0	0.35	0		4.26	0.75	-0.07	-0.06
12	31	7.23	4.03	7.77	0.8	0.03	0.37	0	0	12.52	1.66	0	0.39	0		4.75	0.86	-0.03	0.02
13	54	9.78	2.02	12.26	0.99	0.2	0.52	0	0	14.36	2.09	0.42	0.82	0		2.1	1.1	0.22	0.3
14	76	11.6	2.35	14.45	0.91	0.01	0.3	0	0	17.38	1.95	0.04	0.48	0		2.93	1.04	0.03	0.18
15	96	13.1	3.14	14.68	1.35	0.1	0.2	0	0	17.79	2.32	0.43	0.9	0		3.11	0.97	0.33	0.7
16	120	14.5	2.3	16.34	1.11	0.29	0.55	0	0	18.78	2.34	0.44	0.85	0		2.44	1.23	0.15	0.3
17	146	13.9	1.61	16.38	1.07	0.14	0.5	0	0	18.81	2.47	0.58	1.04	0		2.43	1.4	0.44	0.54
18	175	15.1	2.19	16.61	1.1	0.27	0.55	0	0	18.1	2.34	0.5	0.9	0		1.49	1.24	0.23	0.35
19	192	15.5	1.57	16.68	0.78	0	0.16	0	0	19.2	2.35	0.43	0.94	0		2.52	1.57	0.43	0.78

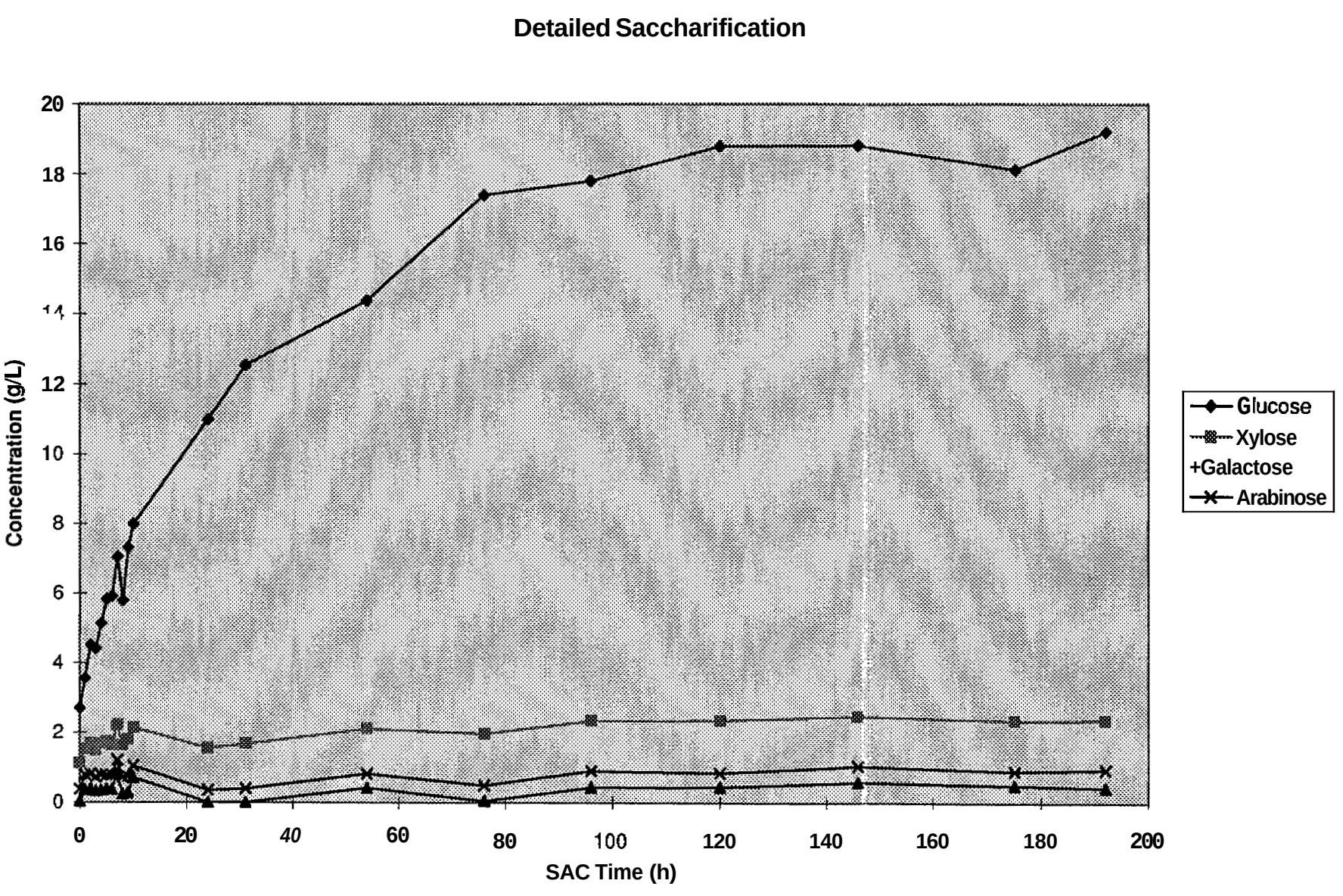

Detailed Saccharification
Glucose Con. YSI (g/L)
SAC Time (h)
0
2
4
6
8
10
12
14
16
0
20
40
60
80
100
120
140
160
180
200


Detailed Saccharification (YSI vs CATLC)
Glucose Concentration (g/L)
0
2
4
6
8
10
12
14
16
18
0
20
40
60
80
100
120
140
160
180
200
SAC Time (h)
Glucose YSI
Glucose CATLC

CAT Task numbers for cellobiose **and** Oligomeric glucose (total-monomeric)



Washed, Corn Fiber Solids Soccharification, Total Sugars as measured **by 4%** hydrolysis CAT



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SSF CARBON BALANCE: Detailed Saccharification of **Washed Corn Fiber- (cat95-090, CAT95-089, 95-095)**

Sample:
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	9.99	21.95
Insoluble Solids (%):	5.00	2.30

Cellulose Conversion:	97.6%
Overall C6-Sugar Conversion:	
Overall C5-Sugar Conversion:	
Ethanol Process Yield (% theor):	
Ethanol Metabolic Yield (% theor):	

Carbon Balance: SSF

	Carbon In							Corbon Out							Conversion	Yield
	In Solids			*In Liquor*			*Total*	*In Solids*			In Liquor			*Total*	*(In-Out)/In*	g product/
Component	(% dry wt)	(C-mole/Kg Sl	(% Total In)	(g/L)	(C-mole/Kg Sl	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sl	% Total Out)	(g/L)	(C-mole/Kg Sl	% Total Out)	(C-mole/Kg Sl	(%)	100 g C6 con
Glucose	**42.7**	**0.711**	*89.3*	2.70	*0.085*	*10.7*	***0.796***	2.22	0.017	*2 6*	19.20	**0.625**	**97.4**	0.642	**19.43**	
Galactose	**0.07**	0.001	*55.1*	0.03	0.001	*44.9*	0.002	0.09	0.001	4 7	**0.43**	0.014	*95.3*	0.015	-594.19	
Mannose	**0.56**	0.009	*100.0*	0.00	0.000	*0.0*	0.009	*0.29*	0.002	*100.0*	0.00	0.000	*0.0*	0.002	76.18	
Xylose	**1.74**	0.029	**45.2**	**111**	0.035	*54.8*	0.064	1.36	**0.010**	*12 0*	**2.35**	0.076	*88.0*	*0.087*	*-35.55*	
Arabinose	**0.38**	0.006	**38.5**	**0.38**	0.012	*65.5*	0.018	0.43	0.003	*100.0*	*0.00*	0.000	*0.0*	0.003	82.05	
Lignin	***9.99***	0.239	*83.6*	1.03	0.047	*16.4*	0.285	21.95	**0.241**	*79 7*	1.32	0.062	**203**	**0.383**	-6.10	
Extractives	*20.M*							**25.90**								
Total C	*32.96*	164.800		**0.46**	6.900		171.700	**33.01**	*75.923*		1.19	21.063		96.986	**171.14**	
Total H	4.71	23.550		**6.71**	100.650		**124.203**	4.39	8.517		*7.25*	130.935		139.452	*123.08*	
Total N	*2.57*	**12.850**		**0.13**	**1.950**		14.800	**5.85**	11.349		**0.15**	**2.709**		14.058	13.85	
% protein	**15.47**							**35.22**								
Total	**5085**	202.196	*64.8*		109.680	*35.2*	***311.876***	*25.87*	*96.064*	*38.2*		155.484	*61.8*	*251.547*		*#REF!*

C- Recovery:	80.66%

SSF CARBON BALANCE: Detailed Saccharification of Washed Corn Fiber (cat95-090, CAT95-089, 95-095

Sample:
Pretreatment:
Run:

SOLIDS BALANCE	In	Out
Lignin (%):	9.99	21.95
Insoluble Solids (%):	5.00	2.30

SLURRY - LIQ #

Cellulose Conversion:	97.6%
Overall C6-Sugar Conversion:	
Overall C5-Sugar Conversion:	
Ethanol Process Yield (% theor):	
Ethanol Metabolic Yield (% theor):	

Carbon Balance: SSF

	Carbon In							Carbon Out							Conversion	Yield
	In Solids			In Liquor			Total	In Solids			In Liquor			Total	(In-Out)/In	g product/
Component	(% dry wt)	(C-mole/Kg Sl	(% Total In)	(g/L)	(C-mole/Kg Sl	(% Total In)	(C-mole/Kg Sln)	(% dry wt)	(C-mole/Kg Sl	% Total Out)	(g/L)	(C-mole/Ka Sl	% Total Out)	(C-mole/Ka Sl	(%)	100 g C6 con
Glucose	42.7	0.711	89.3	2.70	0.085	*10.7*	0.796	2.22	0.017	2.6	19.20	0.625	97.4	0.642	19.43	
Galactose	0.07	0.001	*55.1*	0.03	0.001	*44.9*	0.002	0.09	0.001	*4 7*	0.43	0,014	*95.3*	0.015	*-594.19*	
Mannose	0.56	0.009	*100.0*	*0.00*	0.000	*0.0*	0.009	*0.29*	0.002	*100.0*	0.00	0.000	*0.0*	0.002	76.16	
Xylose	1.74	0.029	*45.2*	1.11	0.035	*54.8*	0.064	1.36	0.010	*12.0*	2.35	0.076	88.0	0.087	-35.55	
Arabinose	0.38	0.006	*34.5*	0.38	0.012	*65.5*	0.016	0.43	0.003	*100.0*	*0.00*	0.000	*0.0*	0.003	82.05	
Lignin	9.99	*0.239*	83.6	1.03	0.047	*16.4*	0.285	21.95	0.241	79.7	1.32	0.062	*20.3*	0.303	-6.10	
Extractives	20.64							25.90	(.33 x 230g)			(.0119 * 1770g)				
Total C	32.96	164.800		046	6.900		171.700	33.01	75.923		1.19	21.063		96.986	171.14	
Total H	4.71	23.550		6.71	100.650		124.200	*4.39*	8.517		7.25	130.935		139.452	123.08	
Total N	2.57	12.850		0.13	1.950		14.800	5.65	11.349		0.15	2.709		14.058	13.85	
% protein	15.47							35.22								
Total	50.85	202.196	*64.8*		109.680	*35.2*	*311.876*	*25.87*	*96.064*	38.2		155.484	61.8	*251.547*		#REF!

Carbon Balance NOT

C recovery:	80.6%

SAC

Based on initial Data - SOLID fraction only, LIQUIDS still under CAT analysis

SSF CARBON BALANCE: Detailed Saccharification of Washec Corn Fiber (cat95-090)

Sample:
Pretreatment:
Run:

Klason + Acid soluble (Extracted samples)

SOLIDS BALANCE	In	Out
Lignin (%):	9.99	21.95
Insoluble Solids (%):	5.00	1.94

measured values

Cellulose Conversion:	98.0%
Overall C6 Sugar Conversion:	82.1%
Overall C5 Sugar Conversion:	-6.9%
Ethanol Process Yield (% theor):	47.7%
Ethanol Metabolic Yield (% theor):	58.1%

98% oligomers Xylose.

Carbon Balance: SSF

Component	Carbon In: In Solids (% dry wt)	(C-mole/Kg Sl	(% Total In)	In Liquor (g/L)	(C-mole/Kg Sl	(% Total In)	Total (C-mole/Kg Sln)	Carbon Out: In Solids (% dry wt)	(C-mole/Kg Sl	(% Total Out)	In Liquor (g/L)	(C-mole/Kg Sl	(% Total Out)	Total (C-mole/Kg Sl	Conversion (In-Out)/In (%)	Yield g product/ 100 g C6 con
Cellobiose				0.03	0.000		0.000				0.00	0.000		0.000		
Glucose	42.7	0.711	55.5	18.05	0.571	64.5	1.282	2.22	0.014	8.2	4.91	0.160	91.8	0.175	86.38	
Galactose	0.07	0.001	0.9	4.12	0.130	99.1	0.132	0W	0.001	0.6	2.81	0.092	99.4	0.092	29.85	
Mannose	0.56	0.009	10.4	2.55	0.081	89.6	0.090	0.29	0.002	100.0	0.00	0.000	0.0	0.002	97.93	
Xylose	1.74	0.029	4.6	19.04	0.603	95.4	0.632	1.36	0.009	1.3	M.39	0.666	98.7	0.675	-6.84	
Arabinose	0.30	0.006	1.7	11.88	0.373	98.3	0.380	0.43	0.003	0.7	12.35	0.403	W.3	0.406	-6.97	
Lignin	9.99	0.239	47.0	5.93	0.269	53.0	0.508	21.95	0.203	42.3	5.92	0.277	57.7	0.480	5.40	
Ethanol				1.00	0.041		0.041				12.20	0.519		0.519		29.70
Cell Mass				0.20	0.008		0.008				2.24	0.088		0.088		5.41
Carbon Dioxide												0.253		0.253		30.08
Glycerol				0.08	0.002		0.002				0.55	0.018		0.018		1.16
Acetic Acid				1.61			0.051				3.02	0.099		0.099		3.86
Lactic Acid				0.49	0.016		0.016				2.92	0.095		om5		6.46
Succinic Acid				0.84	0.027		0.027				1.68	0.056		0.056		2.29
Total	50.85	0.996	31.4		2.172	686	1.168	25.87	0.231	7.8		2.727	92.2	2.958		79.06

need liquids

Lignin balance

N/A no cells

C-RECOVERY: 93.38%

NREL Protected CRADA Information

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-090

Page 1 of 1

Project Title: AMOCO CRADA, Detailed Saccharification (ET60)

NREL In-House ☐ Current Subcontractor ☐ CRADA ☐ Other ☐

Name of Project Contact Person: Tammy Kay Hayward	Date Work Completed: 7/6/95.
NREL Notebook: #1561, p048, #1382, p117	Date Samples Delivered: 6/15/95
Sample Description: Initial and final timepoint solids from Corn Fiber Saccharification.	Actual Hours Spent: 16
Summary of Requested Work: Lyophilization followed by 95% ethanol extraction, complete compositional analysis, and starch analysis.	Proposed Approach: Standard LAPs by validated outside laboratory and by in house analysts.

Work Required: Sample Prep ☐ Acid Digest ☐ HPLC ☐ YSI ☐ GC ☐ Other: 95% ethanol extractions, starch

Results and Comments ☐ % As Received ☐ % Dry Weight ☐ mg/mL ☐ Other:

	Sample		TS	EXT	G	X	GA	A	M	LKL	LAS	AT	MB	ST
	Initial time, Detailed Saccharification, Corn Fiber Solids, 95-090-618	ave	100.00	n/a	53.81	2.19	0.09	0.48	0.70	8.76	3.83	26.79	90.87	0.9
		sd	0.00	—	0.20	0.02	0.00	0.02	0.02	0.23	0.05	0.16	---	*
	Initial time. Corn Fiber Solids, 95-090-618, including extractives	ave	n/a	20.64	42.70	1.74	0.07	0.38	0.56	6.95	3.04	21.16	92.75	0.72
		sd	---	0.27	0.15	0.02	0.00	0.02	0.02	0.18	0.04	0.13	---	*
2	Final Time, Detailed Saccharification, Corn Fiber Solids, 95-090-617	ave	100.00	n/a	2.99	1.84	0.12	0.58	0.39	23.09	6.55	39.23	74.15	0.70
		sd	0.00	—	0.08	0.07	0.00	0.00	0.00	0.47	0.07	0.16	---	0.05
	Final Time, Corn Fiber Solids, 95-090-617, including extractives		n/a	25.90	2.22	1.36	0.09	0.43	0.29	17.11	4.85	29.07	80.85	0.52
			---	0.34	0.06	0.05	0.00	0.00	0.00	0.35	0.05	0.12	---	0.05

A=arabinose; AT=total ash; C=Mass % carbon: EXT=95% ethanol extractives: G=glucose; G-YSI=glucose by YSI; GA=galactose; H=mass % hydrogen; LAS=acid soluble lignin; LKL=Klason lignin; M=mannose; MB=material balance, [EXT + (G+GA+M)x0.90 + (X+A)x0.88 + LKL + LAS + AT]: N=mass % nitrogen: n/a=not applicable: nd=not detected: nr=not requested: ST=starch, % dry weight: TS=total solids: TDS=total dissolved solids; X=xylose; *=Not enough sample to run in duplicate.

Name(s) of CAT Staff Working on Project: Larry Brown, Ray Ruiz

Reviewed by: Tina Ehrman

CC: Christos Hatzis

CHEMICAL ANALYSIS & TESTING

Analysis No. 95-089

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Project Title: Detailed SAC: Work Package ET60

NREL In-House ☐ Current Subcontractor ☐ CRADA ☒ Other ☐

Date Samples Delivered: June 15, 1995

Date Work Promised: n/a

Name of Project Contact Person: Tammy Kay Hayward

Date Work Completed: August 11, 1995

NREL Notebook: #1638 p 055-085

Estimated Hours Required: Not Given

Sample from Feedstock Lot No.: Not Given

Actual Hours Spent: 504

Summary of Requested Work: Sugars pre- and post- 4% acid hydrolysis

Proposed Approach: Standard CAT task analytical methods, and standard LAP's.

Work Required: Sample Prep ☒ NDF/ADF ☐ Acid Digest ☒ HPLC ☒ YSI ☒ GC ☐ Other:

	Sample		CEL	G	X	GA	A	M	YSI-G
1	Detailed Sac 0 (00740) as received	ave	0.54	2.16	0.58	0.07	0.46	0.00	1.84
		sd	0.00	0.02	0.01	0.00	0.01	0.00	0.01
		ave	nd	2.70	1.11	0.03	0.38	0.00	3.00
		sd	--	0.07	0.03	0.00	0.02	0.00	0.07
	Detailed Sac 0 (00738) as received	ave	0.29	2.28	0.64	0.17	0.51	0.00	1.94
		sd	0.00	0.01	0.00	0.00	0.00	0.00	0.01
		ave	nd	2.98	1.53	0.36	0.83	0.00	2.78
		sd	--	0.30	0.07	0.02	0.05	0.00	0.08
3	Detailed Sac 1 (00732) as received	ave	0.74	2.52	0.53	0.13	0.42	0.00	1.43
		sd	0.01	0.00	0.01	0.01	0.01	0.00	0.01
		ave	nd	3.56	1.52	0.35	0.78	0.00	3.43
		sd	--	0.00	0.02	0.04	0.03	0.00	0.03
4	Detailed Sac 2 (00741) as received	ave	0.44	3.37	0.57	0.15	0.43	0.00	2.01
		sd	0.02	0.04	0.02	0.01	0.01	0.00	0.01
	Detailed Sac 2 (00741) after 4% hydrolysis	ave	nd	4.51	1.67	0.38	0.80	0.00	3.18
		sd	--	0.44	0.13	0.06	0.08	0.00	0.03

No values are given for mannose because of poor resolution from another component in the samples. In most cases a small broad peak was observed in the region where mannose elutes, however. the retention time was often significantly longer than that for mannose, as if two components were co-eluting.

Name(s) of CAT Staff Working on Project: P. Ashley, F.P. Eddy, and D. Johnson

Fannie Eddy D. K. Johnson

CAT Task Leader: C. Ehrman

Tina Ehrman

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-089

Results and Comments ☐ % As Received ☐ % Dry Weight ☒ Other: mg/mL

	Sample		CEL	G	X	GA	A	M	YSI-G
5	Detailed Sac 3 (00733) as received	ave	1.23	3.12	0.64	0.17	0.48	0.00	1.71
		sd	0.02	0.02	0.00	0.00	0.01	0.00	0.01
	Detailed Sac 3 (00733) after 4% hydrolysis	ave	nd	4.40	1.48	0.33	0.72	0.00	4.44
		sd	--	0.04	0.02	0.00	0.01	0.00	0.09
6	Detailed Sac 4 (00739) as received	ave	1.06	3.74	0.63	0.16	0.46	0.00	2.17
		sd	0.01	0.00	0.00	0.00	0.03	0.00	0.02
	Detailed Sac 4 (00739) after 4% hydrolysis	ave	nd	5.12	1.66	0.35	0.78	0.00	4.97
		sd	--	0.13	0.09	0.01	0.02	0..00	0.05
7	Detailed Sac 5 (00735) as received	ave	0.63	4.89	0.71	0.19	0.50	0.00	2.90
		sd	0.06	0.06	0.03	0.02	0.02	0.00	0.01
	Detailed Sac 5 (00735) after 4% hydrolysis	ave	nd	5.82	1.71	0.37	0.77	0.00	5.64
		sd	--	0.01	0.02	0.06	0.05	0.00	0.10
8	Detailed Sac 6 (00736) as received	ave	1.20	4.44	0.71	0.19	0.52	0.00	2.91
		sd	0.00	0.12	0.02	0.01	0.01	0.00	0.01
	Derailed Sac 6 (00736) after 4% hydrolysis	ave	nd	5.91	1.63	0.37	0.77	0.00	5.97
		sd	--	0.02	0.02	0.01	0.01	0.00	0.02
9	Detailed Sac 7 (00731) as received	ave	1.34	4.80	0.75	0.12	0.47	0.00	3.01
		sd	0.03	0.08	0.02	0.01	0.01	0.00	0.03
	Detailed Sac 7 (00731) after 4% hydrolysis	ave	nd	7.03	2.20	0.82	1.21	0.00	6.45
		sd	--	0.43	0.30	0.24	0.27	0.00	0.02
10	Detailed Sac 8 (00725) as received	ave	1.56	3.50	0.65	0.17	0.50	0.00	2.61
		sd	0.05	0.03	0.01	0.00	0.00	0.00	0.04
	Detailed Sac 8 (00725) after 4% hydrolysis	ave	nd	5.78	1.64	0.24	0.70	0.00	5.75
		sd	--	0.15	0.10	0.05	0.05	0.00	0.06
11	Detailed Sac 9 (00727) as received	ave	1.74	4.97	0.75	0.18	0.50	0.00	3.51
		sd	0.11	0.37	0.09	0.02	0.03	0.00	0.00
	Detailed Sac 9 (00727) after 4% hydrolysis	ave	nd	7.31	1.78	0.27	0.73	0.00	7.27
		sd	--	0.07	0.01	0.00	0.00	0.00	0.15

A=arabinose; AC=acetate; ASL=acid-soluble lignin; CEL=cellobiose; ET=ethanol; FL=furfural; G=glucose; GA=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LAC=lacticacid; M=mannose; nd=notdetected; TS=wieght%totalsolids; TDS=weight%totaldissolvedsolids; X=xylose; YSI-G=Glucose determined by YSI

values are given for mannose because of poor resolution from another component in the samples. In most cases a small broad peak was observed in the region where mannose elutes, however, the retention time was often significantly longer than that for mannose, as if two components were co-eluting.

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-089

Results and Comments ☐ % As Received ☐ % Dry Weight ☒ Other: mg/mL

Sample		CEL	G	X	GA	A	M	YSI-G
12 Detailed Sac 10 (00728) as received	ave	3.08	4.54	0.84	0.15	0.50	0.00	3.47
	sd	0.04	0.19	0.03	0.01	0.00	0.00	0.01
Detailed Sac 10 (00728) after 4% hydrolysis	ave	nd	7.99	2.13	0.70	1.04	0.00	7.54
	sd	--	0.15	0.06	0.04	0.04	0.00	0.04
13 Detailed Sac 11 (00726) as received	ave	3.42	6.71	0.79	0.07	0.41	0.00	5.97
	sd	0.05	0.11	0.07	0.06	0.08	0.00	0.01
Detailed Sac 11 (00726) after 4% hydrolysis	ave	nd	10.97	1.54	0.00	0.35	0.00	11.14
	sd	--	0.02	0.01	0.00	0.02	0.00	0.04
14 Detailed Sac 12 (00619) as received	ave	4.03	7.77	0.80	0.03	0.37	0.00	7.09
	sd	0.04	0.03	0.02	0.02	0.01	0.00	0.01
Detailed Sac 12 (00619) after 4% hydrolysis	ave	nd	12.52	1.66	0.00	0.39	0.00	12.55
	sd	--	0.01	0.04	0.00	0.03	0.00	0.18
15 Detailed Sac 13 (00737) as received	ave	2.02	12.26	0.99	0.20	0.52	0.00	11.25
	sd	0.01	0.03	0.01	0.01	0.01	0.00	0.07
Detailed Sac 13 (00737) after 4% hydrolysis	ave	nd	14.36	2.09	0.42	0.82	0.00	13.62
	sd	--	0.42	0.12	0.05	0.06	0.00	0.14
16 Detailed Sac 14 (00730) as received	ave	2.35	14.45	0.91	0.01	0.30	0.00	12.88
	sd	0.06	0.01	0.00	0.00	0.05	0.00	0.06
Detailed Sac 14 (00730) after 4% hydrolysis	ave	nd	17.38	1.95	0.04	0.48	0.00	17.55
	sd	--	0.11	0.01	0.00	0.00	0.00	0.19
17 Detailed Sac 15 (00734) as received	ave	3.14	14.68	1.35	0.10	0.20	0.00	13.40
	sd	0.18	0.11	0.32	0.14	0.00	0.00	0.14
Detailed Sac 15 (00734) after 4% hydrolysis	ave	nd	17.79	2.32	0.43	0.90	0.00	17.53
	sd	--	0.13	0.02	0.00	0.00	0.00	0.07
18 Detailed Sac 16 (00722) as received	ave	2.30	16.34	1.11	0.29	0.55	0.00	15.71
	sd	0.19	0.15	0.07	0.07	0.11	0.00	0.13
Detailed Sac 16 (00722) after 4% hydrolysis	ave	nd	18.78	2.34	0.44	0.85	0.00	17.85
	sd	--	0.07	0.00	0.02	0.01	0.00	0.54

A=arabinose; AC=acetate; Askacid-soluble lignin; CEL=cellobiose; ET=ethanol; FL=furfural; G=glucose; GA=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LAC=lacticacid; M=mannose; nd=notdetected; TS=wieght%totalsolids; TDS=weight%totaldissolvedsolids; X=xylose; YSI-G=Glucose determined by YSI

values are given for mannose because of poor resolution from another component in the samples. In most cases a small broad peak was observed in the region where mannose elutes, however, the retention time was often significantly longer than that for mannose, as if two components were co-eluting.

CHEMICAL ANALYSIS & TESTING
(CAT) Task Analytical Report

Analysis No. 95-089 | Page 4 of 5

Results and Comments ☐ % As Received ☐ % Dry Weight ☒ Other: mg/mL

	Sample		CEL	G	X	GA	A	M	YSI-G
19	Detailed Sac 17 (00729) as received	ave	1.61	16.38	1.07	0.14	0.50	0.00	15.78
		sd	0.01	0.07	0.00	0.00	0.00	0.00	0.11
	Detailed Sac 17 (00729) after 4% hydrolysis	ave	nd	18.81	2.47	0.58	1.04	0.00	19.11
		sd	--	0.14	0.01	0.03	0.01	0.00	0.07
20	Detailed Sac 18 (00724) as received	ave	2.19	16.61	1.10	0.27	0.55	0.00	16.33
		sd	0.08	0.10	0.03	0.03	0.04	0.00	0.07
	Detailed Sac 18 (00724) after 4% hydrolysis	ave	nd	18.10	2.34	0.50	0.90	0.00	17.31
		sd	--	0.27	0.03	0.03	0.01	0.00	0.46
21	Detailed Sac 19 (00723) as received*	ave	1.57	16.68	0.78	0.00	0.16	0.00	16.35
		sd	0.14	0.19	0.15	0.00	0.13	0.00	0.30
	Detailed Sac 19 (00723) after 4% hydrolysis	ave	nd	19.20	2.35	0.43	0.94	0.00	18.26
		sd	--	0.03	0.01	0.00	0.00	0.00	0.11

A=arabinose; AC=acetate; ASL=acid-soluble lignin; CEL=cellobiose; ET=ethanol; FL=furfural; G=glucose; GA=galactose; GLY=glycerol; HMF=5-hydroxymethyl-2-furaldehyde; LAC=lacticacid; M=mannose; nd=notdetected; TS=wieght%totalsolids; TDS=weight%totaldissolvedsolids; X=xylose; YSI-G=Glucose determined by YSI

* Repeated analyses of sample 00723 gave somewhat variable results. Material was seen togrow relatively rapidly in this sample. The results from the first analysis completed after the analytical sample was prepared.

No values are given for mannose because of poor resolution from another component in the samples. In most cases a small broad peak was at region where mannose elutes, however, the retention time was often significantly longer than that for mannose. as if two components were co-e

CHEMICAL ANALYSIS & TESTING
(CAT) Task Analytical Report

Analysis No. 95-089

NREL Notebook: #1383 p 062

Estimated Hours Required: Not Given

Summary of Requested Work: CHN Analysis

Proposed Approach: Standard CAT task analytical methods

	Sample		C	H	N				
1	Detailed Sac 0 (00740) as received	ave	0.46	6.71	0.13				
		sd	0.01	0.02	0.01				
2	Detailed Sac 19 (00723) as received	ave	1.19	7.25	0.15				
		sd	0.05	0.17	0.03				

Name(s) of CAT Staff Working on Project: R. Ruiz

CAT Task Leader: C. Ehrman

Toni Ehrman

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-095 | Page 1 of 1

Project Title: Amoco CRADA, Detailed Saccharification (ET60)

NREL In-House	Current Subcontractor	CRADA	Other	Date Samples Delivered: 7-16-95
☐	☐	☒	☐	Date Work Promised: ASAP

Name of Project Contact Person: T.K. Hayward

Date Work Completed: 8-18-95

NREL Notebook: 1382 p118, 1383 p62

Estimated Hours Required: n/a

Samples from Feedstock Lot No.: n/a

Actual Hours Spent: 5

Summary of Requested Work: Acid soluble lignin, carbon, hydrogen. nitrogen, and protein determination.

Proposed Approach: Standard CAT Task methods of analysis.

Work Required:	Sample Prep	NDF/ADF	Acid Digest	HPLC	YSI	GC	Other:
	☐	☐	a	a	a	☐	CHN

sample description box

	Detailed saccharification, Corn Fiber Liquids. T 19, 95-089-723 (mass% as recvd for CHNP) (mg/mL for LAS)	ave	1.19	7.25	0.15	***	1.03		
		sd	0.05	0.17	0.03	—	0.01		
2	Detailed saccharification, Corn Fiber Liquids, T zero, 95-089-740 (mass% as recvd for CHNP) (mg/mL for LAS)	ave	0.46	6.71	0.13	***	1.32		
		sd	0.01	0.02	0.01	—	0.01		
3	Detailed saccharification, lyophilized and extracted, Corn Fiber Solids. T initial, 95-090-618 (mass % dry wt. for CHNP)	ave	32.96	4.71	2.57	15.47	n/a		
		sd	0.09	0.04	0.05	—	—		
4	Detailed saccharification, lyophilized and extracted. Corn Fiber Solids. T final, 95-090-617 (mass % dry wt. for CHNP)	ave	33.01	4.39	5.85	35.22	n/a		
		sd	0.19	0.08	0.05	—	—		

* = Samples listed reference those submitted as part of earlier sample groups (95-089 & 95-090).
** = Percentage protein determined by *mass* %N x 6.02, based on amino acid profiles for this sample type and prep.
*** = No conversion factor for protein. from maw %N, available for liquor samples.

Name(s) of CAT Staff Working on Project:
Ray Ruiz Ray Ruiz
Larry Brown Larry Brown

CAT Task Leader:
Tina Ehrman Tina Ehrman

CHEMICAL ANALYSIS & TESTING (CAT) Task Analytical Report

Analysis No. 95-106

Project Title: *Amoco* CRADA - **HPAEC-PAD** Analysis of Selected Detailed Saccharification Samples (ET60)

NREL Ir-House ☐ Current Subcontractor ☐ **CRADA** ☒ Other ☐

Name of Project Contact Person: Tammy Kay Hayward

Date **Work** Completed: ***8/25/95***

NREL **Notebook: #1181, page** 118

Date Samples Delivered: 8/21/95 (following completion of project 95-089)

Samples from Feedstock Lot No.: *Amoco* **corn** fiber

Actual Hours Spent: **8** hours

Summary of Requested Work: Samples had previously been **analyzed** for soluble **and** total sugars (refer to CAT **Task** report 95-089). Two **samples** were selected for **analysis** by **HPAEC-PAD** to resolve problems **with** coeluting components noted in **the** conventional **HPLC** approach.

Proposed Approach: HPAEC-PAD by the validated special projects method.

Work Required: Sample Prep ☐ Acid Digest ☐ HPLC ☒ **YSI** ☒ *GC* ☐ Other: HPAEC-PAD

Results and Comments ☐ % **As** Received ☐ % Dry Weight ☒ mg/mL ☐ Other:

	Sample**		G	X	GA	A	M				G-YSI
1	Detailed **saccharification liquor #14, as** received (95-089-730)	HPX-87P	14.45	0.91	0.01	0.30	nd				12.88
		HPAEC-PAD	**12.62**	**0.97**	0.12	**0.57**	nd				n/a
2	Detailed **saccharification liquor #16, as received (95489-722)**	**HPX-87P**	**16.34**	1.11	**0.29**	**0.55**	nd				**15.71**
		HPAEC-PAD	**15.46**	**1.07**	0.26	0.59	nd				n/a

A = arabinose; G = glucose; G-YSI = glucose by **YSI;** GA = galactose; M = mannose; n/a = not applicable: nd = not detected; X = xylose

"NOTE: the samples selected for further **analysis by** HPAEC-PAD **had** produced complicated baselines by the conventional **HPLC** approach **(using** the HPX-87P column), making it difficult to resolve the **sugars from** other components eluting **as part** of **the** "disturbed" baseline: **this** lead to a slight overestimation of glucose; the **HPAEC-PAD** approach **did** not **suffer** from **this** coelution problem, producing glucose **values that** were in better agreement with the YSI glucose values. Good agreement **was obtained** between **HPAEC-PAD and** HPX-87P **runs** with **the** other **sugars** of **interest.**

Name(s) of **CAT Staff** Working on Projecr: Tina Ehrman

Tina Ehrman

Reviewed by: Ray Ruiz

Ray Ruiz